The effect of Hydro-alcoholic extract of pumpkin seeds on Oogenesis and Hormone-Pituitary-Ovarian Axis in Mature Rats

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Original Article

Abstract

Introduction: In traditional medicine, pumpkin seeds are prescribed and used commonly as energetic and sexual enhancer substances. This study was planned and carried out in order to fill the gap caused by the lack of sufficient scientific evidence on the effect of this substance on oogenesis and hormone-pituitary-ovarian axis. Revealing the effect of hydro-alcoholic extract of pumpkin seeds on oogenesis and hormone-pituitary-ovarian axis of mature rats.

Methods: In this experimental study a number of 30 mature female Wistar rats (weight range: 180±10) were tested. They were divided randomly into four 6-member groups, including three experiment and a control groups. Members of experimental groups were injected intraperitoneally by the extract (with 50, 100, and 200 mg/kg of hydro-alcoholic extract of pumpkin seeds) for 21 consecutive days. A day after the last injection, the rats' blood was taken for analyzing the sexual hormones and ovaries were dissected for histological studies.

Results: Injecting hydro-alcoholic extract of pumpkin seeds increases significantly the serum level of estrogen, progesterone, LH, FSH, number of graafian follicles and also decreases significantly the left ovaries' weight and number of primordial, primary, and secondary follicles in experimental groups in contrast to control group ($P<0.05$).

Conclusion: Injecting hydro-alcoholic extract of pumpkin seeds increases significantly the oogenesis procedure, LH, FSH, estrogen and progesterone levels.

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Introduction:

Generally, reproductive system in mammals has two important tasks: producing reproductive/sex cells (gametes) and producing sex hormones (1). The female reproductive system is tasked with producing female gametes and also developing and maintaining them both before and after fertilization period (2). Female sex cells (female gametes) are produced in ovaries where estrogen and progesterone (female sex hormones) are secreted (3). The puberty is associated with some certain

physical and mental developments including physical developments along with hypothalamus-pituitary - gonad axis (4). For example, the menopause cycle begins along with the growth and puberty of follicles, which are controlled by FSH and estrogen (5,6).

The nature is the rich source of medicinal compounds which some of them are found in plants; as these plants were being used as traditional medicines since thousands years ago. Recently, in response to side effects of synthetic drugs, many developing countries have started to use plant medicines to treat various diseases, many food factories have started to employ natural oxidants and many studies on extracting biologic active compounds from plants are being carried out now (7). Pumpkin and its seeds are popular foods for American Indians and have unique health and nutritional properties. Pumpkin seeds are used in some European cuisines, especially in southern parts of Austria, Hungary, and Slovenia's. Today, pumpkin is produced commercially in the United States, Mexico, India and China (8).

Pumpkin belongs to Cucurbitaceae family (9). In this study *Cucurbita* maxima species was used. *C. maxima* is an orange colored pumpkin which is more useful in contrast to other species (10). The pumpkin seeds are dark green flat seeds which are encapsulated in a white shell (11). Spain and the South America region are the origin of genetic modification and agricultural diversity of C. maxima (12). Pumpkin seeds not only have proteins, vitamins and antioxidants, but also they contain carotenoids and tocopherols (13).

According to the traditional medicine, consuming pumpkin seeds will alleviate dysuria, stomach ulcer, bloody sputa and coughs (10). Raw pumpkin seeds are used as an anthelmintic (an agent used to expel intestinal worms) and also are useful in treating prostate cancer and prostatitis. Pumpkin seed oil is helpful for treating fever and cramps. Pumpkin and its seeds are very useful to prevent lung cancer (14). This plant indicates a useful source of nutrients for human being (15).

Gossell et al. (2006) observed that pumpkin seeds are full of phytoestrogens which are precursors of estradiol, as its consumption will increase estradiol level (17). Abd El-Ghany et al. (2010) has pointed to daily usage of pumpkin seeds as a part of the diet prescribed for improving the sexual health (16).

Shamloul (2010) reported that despite the increased tendency to use medicinal plants to improve sexual activity and erectile dysfunctions, there is not sufficient evidence to verify their impacts (18).

Pumpkin seeds contain 94 gr. Water, 1.1 gr. Protein, 0.1 gr lipid, 6.3 gr. Starch, 28 mgr. Ca, 30 mgr. phosphorus, 0.4 mgr. iron, 2.2 mgr. potassium, 400 units Vitamin A, 0.05 mgr. Vitamin B1, 0.09 mgr. vitamin B2, 1 mgr. vitamin B3, 22 mgr. vitamin C and also is a good source of Unsaturated fatty acids and phytosterols (β-sitosterol). Pumpkin seeds contain fatty acids such as palmitic acid, stearic acid, oleic acid and linoleic acid, proteins, vitamins, antioxidants, carotenoids, and tocopherols (28). It has been demonstrated that these compounds have positive effects on body tissues. This study is necessary for analyzing the effect of hydro-alcoholic extract of pumpkin seeds on oogenesis and pituitary-ovaries axis.

This study was carried out in order to analyze effect of hydro-alcoholic extract of pumpkin seeds on oogenesis and pituitary-ovaries axis on mature rats.

Methods:

Type of Study: As a randomized experimental trial, this study was carried out on female Wistar rats.

Hydro-alcoholic extract (80%) of pumpkin seeds was prepared as follows:

For extraction Soxhlet extraction technique was used in which 100 gr dried pumpkin seeds was powdered and was mixed with 500 ml ethanol (80%) and was kept in lab condition in a percolator for three days. After three days the extract was collected through the valve in the bottom of the percolator and then ethanol 80% was added to percolator until the collected extract is seen colorless and it indicates that there is no extract; after this phase the resulted mixture was filtered from a filter paper for making it transparent. Then the extract was evaporated in the rotary set at 40° C in order to reach a concentrated extract. Using desiccator, the resulted extract was maintained and

desiccated under a strong vacuum for 24 hours. The final dried extract was weighted and its outcome was measured. Therefore after passing the mentioned phases 16 gr. dried extract was collected from 100 gr. pumpkin powder; then the resulted extract is 16%. Eventually, using LD50 method, the minimum, average and maximum values of pumpkin extract was calculated and then the lethal dose of the medicine was determined and then the minimum, average and maximum doses were injected (19).

Setting dose of pumpkin extract:

Several concentrations of pumpkin extract were selected randomly and were injected to five 6-member groups of rats. The group in which a half of rats were alive and another half were dead (after injection) was considered as the LD50 concentration and then accordingly the LD50 concentration (400 mg/kg) the maximum, average and minimum concentrations were determined. For determining the maximum dose, 200 mg pumpkin seed extract powder, which is half of the LD50 amount, and for the average dose, 100 mg/kg and for the minimum dose, 50 mg/kg pumpkin seed extract were separately dissolved in 1 ml distilled water.

Animals and Grouping

In this study, 30 mature female Wistar rats with the weight range of 180±10 gr. were examined. The rats were prepared from Islamic Azad University Lab Animal Breeding House, Jahrom Branch. The animals were provided with enough water and food during the study period and a circle including 12 hours of daylight and 12 hours of darkness. The rats were kept for two weeks for making them adaptable. All ethical issues involved in working with lab animals were observed.

Thirty female immature Wistar rats were divided randomly into four 6-member groups including 1, 2, and 3 experimental groups and control group. The experimental group received 0.1 ml of 50, 100 and 100 mgr/kg of body weight of pumpkin seed extract; whereas the control group received 0.1 ml physiological saline intraperitoneally for 21 consecutive days.

Co-cycling mature female rats:

All mature female rats need to be positioned in a single sexual cycle for conducting experiments. In rats, estrus cycle lasts for 4-5 days.

Since rats used in this study were in different phases of estrus cycle, we needed a method to regulate all of them in a similar phase of estrus cycle. Vaginal smear was prepared from all rats in order to both determine their cycle and to co-cycle them.

Blood sampling and hormonal experiments

A day after completion of the 21-day course, all animals were anesthetized and were blood samples from their hearts. The collected samples were transferred to centrifuge and the resulted serum was collected with Pasteur pipette. Serums were frozen in -20°C and then were kept in fridge for 48 hours.

Levels of Hormones were measured using ELISA technique and special kit designed for assessing estrogen and progesterone (made by German DRG company) and FSH and LH hormonal kits (made by Iranian Pishtaz Teb Company).

Histological Analysis of Ovary Tissue

After blood sampling, a cut was made in the abdominal area of rats and their ovaries were dissected from the surrounding adipose tissue and Fallopian tube using scalpel and forceps. All animals' ovaries were removed and after weighting by a digital scale, they were washed with physiological saline; then ovaries were put in a glass containing formaldehyde 3% and were kept for 14 days. Ovaries were sent to the histology lab of Shiraz MRI Hospital for preparing slides. It is necessary to say that Hematoxylin-eosin staining was used to prepare slides.

Slides prepared from different parts of ovaries made studying of ovaries' tissues possible. Using an optical microscope with a 400x magnification, each slide was examined in terms of level of hyperemia and congestion, vacuolization of ovarian tissue cells, follicular atresia, the average amount of primordial, primary and secondary, graafian and atretic follicles and the corpus luteum and number of follicles was counted. Then the average number of follicles was determined and was compared with other groups.

Statistical Analysis

Data collected from hormonal assessment and weighting ovaries were analyzed using SPSS 17, one-way analysis of variance (ANOVA) and Tukey's range test (pairwise comparison. The significance level was considered less than 0.05.

Results:

Results gained from measuring the final weights of mature rats indicate that the experimental groups did not show any significant variation in contrast to the control group ($P<0.05$). Figure 1 shows the comparison of final weights of mature rats after injecting pumpkin seed extract to all groups.

Results gained from measuring weight of left ovaries of mature rats in all groups suggested that the this item decreased significantly in experimental groups of 1, 2, and 3 (treating with minimum, mean and maximum doses) in contrast to control group ($P<0.05$). Figure compares the left ovary's weight in mature rats after injecting pumpkin seed extract to all groups.

Results gained from measuring level of FSH in various groups showed that this item decreased significantly in group 3 (maximum dose) in contrast to groups 1 and 2 and the control group ($P<0.05$). Figure 3 compares level of FSH in mature rats after injecting the pumpkin seed extract in various groups.

Results gained from measuring level of LH in various groups showed that this item decreased significantly in group 3 (maximum dose) in contrast to groups 1 and 2 and the control group ($P<0.05$). Groups 1 and 2 did not show any significant variation in contrast to control. Figure 4 compares level of LH in mature rats after injecting the pumpkin seed extract in various groups.

Results gained from measuring level of estrogen in various groups showed that this item decreased significantly in group 3 (maximum dose) in contrast to groups 1 and 2 and the control group. Groups 1 and 2 did not show any significant variation in contrast to control ($P<0.05$). Figure 5 compares level of estrogen in mature rats after injecting the pumpkin seed extract in various groups.

Results gained from measuring level of progesterone in various groups showed that this item decreased significantly in group 3 (maximum dose) in contrast to groups 1 and 2 and the control group. Groups 1 and 2 did not show any significant variation in contrast to control ($P<0.05$). Figure 6 compares level of progesterone in mature rats after injecting the pumpkin seed extract in various groups.

Results gained from counting the number of primordial follicles of ovaries in various groups showed that the average number of these follicles in groups 1, 2, and 3 decreased significantly in contrast to control group ($P<0.05$). Results gained from counting the number of primary follicles of ovaries in various groups showed that the average number of these follicles in groups 1, 2, and 3 decreased significantly in contrast to control group. Results gained from counting the number of secondary follicles of ovaries in various groups showed that the average number of these follicles in groups 1, 2, and 3 did not decrease significantly in contrast to control group ($P<0.05$). Results gained from counting the number of graafian follicles of ovaries in various groups showed that the average number of these follicles in groups 3 increased significantly in contrast to groups 2, and 3 and control group. Groups 1 and 2 did not any significance difference with the control group. Figures 7, 8, 9, and 10 compare number of ovarian follicles in mature rats after injecting pumpkin seeds extract to all groups.

The results gained from counting the corpus luteum in ovaries of various groups show that there was not a significant difference between experimental groups and the control group ($P<0.05$). Figure 11 compares the number of the corpus luteum in ovaries of mature rats after injecting pumpkin seed extract to various groups.

The results gained from counting the atretic follicles in ovaries of various groups show that there was not a significant difference between experimental groups and the control group ($P<0.05$). Figure 12 compares the number of atretic follicles in ovaries of mature rats after injecting pumpkin seed extract to various groups.

Figures of ovarian follicles in various groups of mature rats:

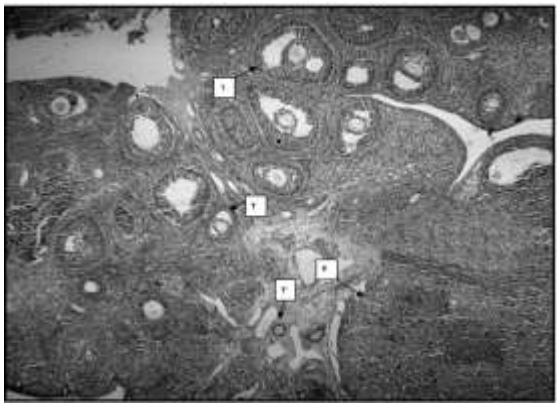

Figure 1. Ovarian follicles in control group of mature rats

Figure (1) photomicrograph of graafian follicle (1), secondary follicle (2), primary follicle (3), the corpus luteum (4), in control group of mature rats with a 40x magnification and Hematoxylin-eosin staining.

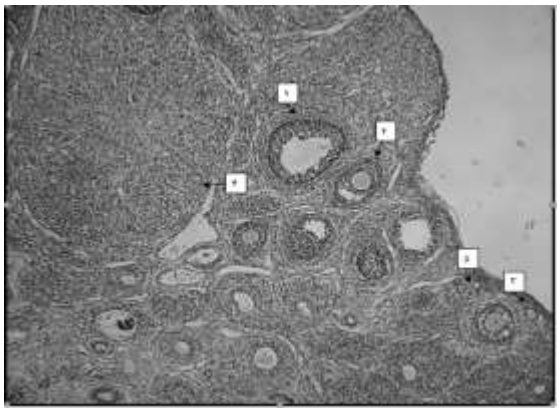

Figure 2. Ovarian follicles in case group of mature rats

No significant difference with control group was seen.

Figure (2) photomicrograph of graafian follicle (1), secondary follicle (2), primary follicle (3), the corpus luteum (4), primordial follicle (5), in case group of mature rats with a 40x magnification and Hematoxylin-eosin staining.

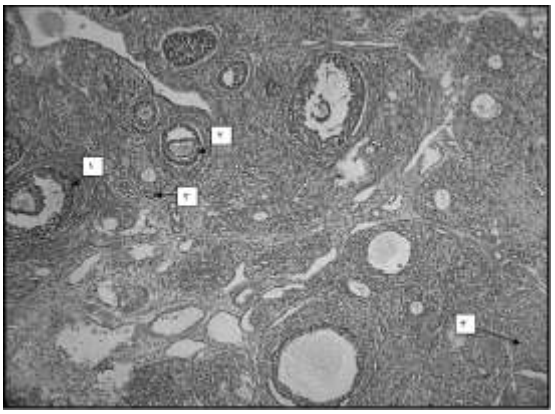

Figure 3. Ovarian follicles in experimental group 1 of mature rats

A significant decrease in number of both primordial and primary follicles in comparison to number of those in control group was seen.

Figure (3) photomicrograph of graafian follicle (1), secondary follicle (2), primary follicle (3), the corpus luteum (4), in experiment group 1 of mature rats (receiver of the minimum dose of pumpkin seed extract) with a 40x magnification and Hematoxylin-eosin staining.

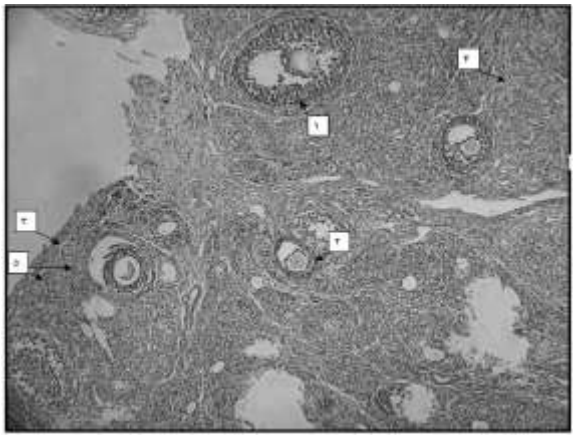

Figure 4. Ovarian follicles in experimental group 2 of mature rats

A significant decrease in number of both primordial and primary follicles in comparison to number of those in control group was seen.

Figure (4) photomicrograph of graafian follicle (1), secondary follicle (2), primary follicle (3), the corpus luteum (4), primordial follicle (5), in experiment group 2 of mature rats (receiver of the

mean dose of pumpkin seed extract) with a 40x magnification and Hematoxylin-eosin staining.

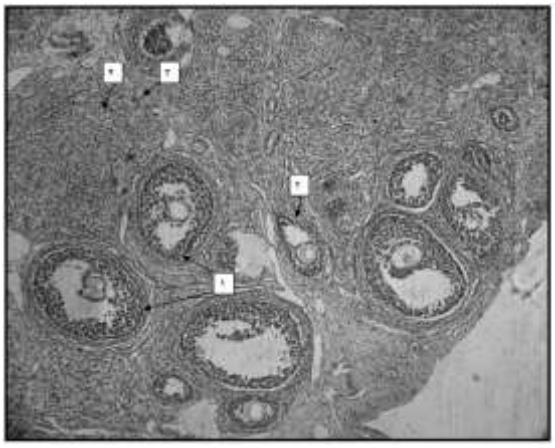

Figure 5. Ovarian follicles in experimental group 3 of mature rats

A significant increase in number of graafian follicles and a significant decrease in number of both primordial and primary follicles in comparison to those in control group were seen.

Figure (5) photomicrograph of graafian follicle (1), secondary follicle (2), primary follicle (3), the corpus luteum (4), in experiment group 3 of mature rats (receiver of the maximum dose of pumpkin seed extract) with a 40x magnification and Hematoxylin-eosin staining.

Figure 1: Results of measuring final weight of mature rat:

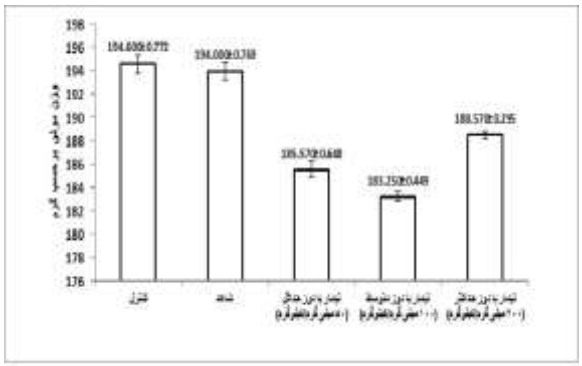

Figure 1. Comparing final weight of mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

Figure 2. Results of measuring left ovarian weight of mature rats

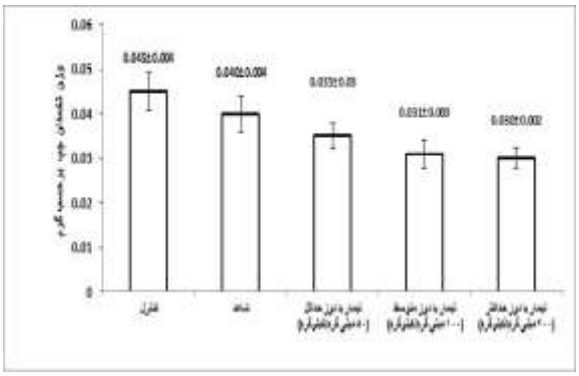

Figure 2. Comparing left ovarian weight of mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

* Experimental groups 1, 2, and 3 showed a significant decrease in contrast to control group

Figure 3. Results of analyzing FSH in mature rats

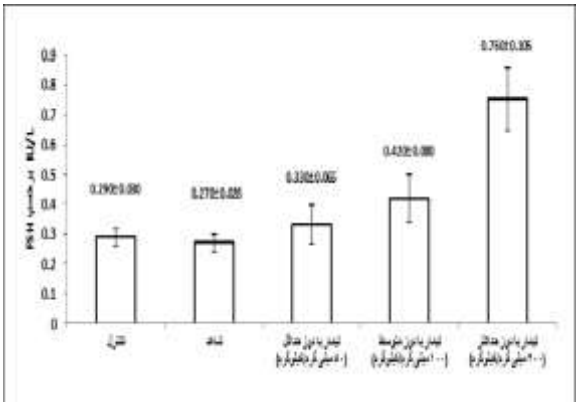

Figure 3. Comparing FSH level in mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

* Experimental groups 3 showed a significant increase in contrast to experimental groups 1 and 2 and control group.

Figure 4. Results of analyzing LH in mature rats

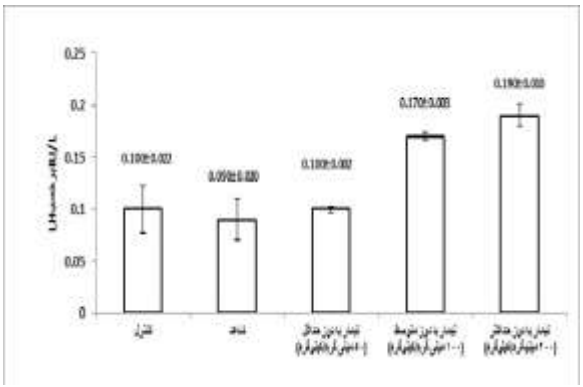

Figure 4. Comparing LH level in mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

* Experimental groups 3 showed a significant increase in contrast to control group.

Figure 5. Results of analyzing estrogen level in mature rats

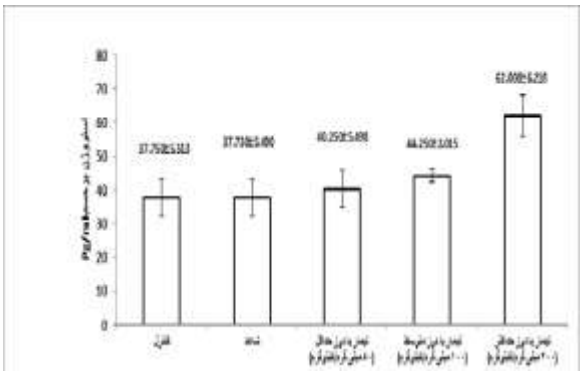

Figure 5. Comparing estrogen level in mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

* Experimental groups 3 showed a significant increase in contrast to control group.

Figure 6. Results of analyzing progesterone level in mature rats

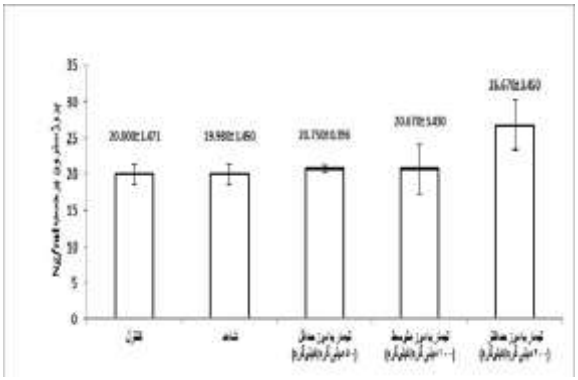

Figure 6. Comparing progesterone level in mature rat after injecting pumpkin seeds extract to various groups (P < 0.5)

Conclusion:

The results of this study showed that injecting hydro alcoholic extract of pumpkin seed increases level of estrogen, LH, FSH hormones and number of graafian follicles, it also decrease significantly weight of left ovaries and number of primordial, primary and secondary follicles in mature rats. It is the first experimental study on mature rats which represents the effect of pumpkin seed on oogenesis and pituitary-ovarian axis. Gossell-Williams et al. (2011) studied on postmenopausal women and demonstrated that pumpkin seeds are full of phytoestrogens which in turn can alleviate main symptoms of menopause such as hot flashes, arthralgia and headache (17). Raicht et al. (1980) and Awad et al. (2000) indicated that pumpkin seeds contain beta estradiol which has many biological effects including reducing cholesterol level, estrogen activity and anti-cancer activity (20,21). This study indicated the significant increase of estrogen in the experiment group received 50 and 100 mg/kg of bodyweight ($P < 0.05$). It was compatible with the results of previous studies. Phytoestrogens are divided into two groups: 1) flavonoids and 2) non-flavonoids. Gossell-Williams et al. (2011) demonstrated that pumpkin seeds are full of phytoestrogens which in turn can alleviate main symptoms of menopause such as hot flashes, arthritis (due to lack of estrogen's parathyroid activity) and headache (7). The results of our study showed the significant increase of estrogen level in mature rats treated with the maximum dose in

contrast to that in control group. Since increasing level of LH enhances synthesis of endogens (22), and this study LH level in experiment group 3 (maximum dose) has increased significantly in contrast to that in control group, the increased level of progesterone is justifiable. For conducting meiosis cell division and achieving the growth capacity and growth potential, ovules need a sufficient level of both LH and FSH (23). Raicht et al. (1980) and Awad et al. (2000) indicated that pumpkin seeds contain phytoestrogens which they in turn have estrogenic activity (24,25). Since increasing estrogen and progesterone has a negative feedback on LH and FSH level (26), although in our study the member of group 3 (receivers of maximum dose) showed a significant increase in progesterone level, a significant increase in FSH level was seen in this group. Maybe it is possible to justify the increased level of FSH in group 3 with Tayne et al. (2002) results; because they indicated that consuming pumpkin seed increases significantly the level of serum LH and FSH in people who have consumed food enriched with pumpkin seeds (27). Further studies on this issue are necessary.

In this study, all groups did not a significant difference with control group in terms of LH level, only the group received maximum dose (experiment group 3), mature rats showed a significant increase in contrast to the control group. It was compatible with Bataineh et al. results; but was not compatible with Tamanini et al. (2003) on the relation between linoleic acid and LH level. Since linoleic acid is one of major fatty acids in pumpkin seeds (28), conjugated linoleic acid (CLA) reduces LH level, as CLA decreases synthesis of leptin. Regarding the absolute and completely significant relationship between leptin and nitric oxide in releasing LH from pituitary gland, the decreased level of LH will result in reduction of nitric oxide and hence the decreased release of GnRH (29).

In this study, a significant reduction was seen in number of ovarian primordial and primary follicles in various mature groups in contrast to those in control group. However, these results are incompatible with the results of previous studies; it seems that the differences are due to a number of important factors such as race of tested animals, level of hormones and treatment duration. Perhaps, such difference is due the fact that pumpkin contains a considerable amount of antioxidants, tocopherols and carotenoids. Therefore, pumpkins potentially have antioxidant activity (30). Kim et al. (2008) studied about side and unexpected effects of antioxidants and concluded that active oxidants in ovarian follicles are necessary for ovulation response before its beginning and reducing free oxygen elements in ovaries inhibits ovulation and a complete set of necessary responses before ovulation (28).

This study also showed the significant increase of ovarian graafian follicles in experimental group 3 in contrast to that in control group, which was compatible with results if Forouzanfar et al. (2005).

Consuming pumpkin seeds will result in considerable enhancement in weight of those who used to foods enriched with pumpkin seeds (27).as Lephart et al (2003) suggested, semi-estrogenic molecules, such as phytoestrogens, have complicated effects on non-reproductive behaviors, such as anxiety and movement. Thus, they can result in anti-anxiety effects which in turn enhance locomotive activity and exploratory behavior and these effects often are related to hormonal action and sexual steroids and the increased motion usually is followed by weight loss (31). Maybe, no weight increase is seen as the result of more movement in experimental groups which it needs further studies.

Raicht et al. (1980) and Awad et al. (2000) showed that pumpkin seeds contain phytoestrogens which have many biological effects such as estrogenic activity (24,25). Manaal et al. (2006) studied about male rats and analyzed the effects of pumpkin seeds on prostate weight, the prostate-binding protein (PBP) and testicular tissue and it was concluded that pumpkin seeds can inhibit hyperplasia in abdominal lobe of prostate only in 10% of cases and also plays an important role in decrease the PBP level and improving the testicular tissue (32). Although, no similar study has been conducted on female rats, regarding Jefferson et al. (2006) we know that there is a relationship between ovary weight and total bodyweight and the increased procedure of folliculogenesis (33). Since in this study no weight increase was seen in mature rats, and its reasons were discussed, the decreased weight of left ovary in experimental groups in

contrast to that in control group was seen. However, conducting further studies on this issue is necessary. Several other studies on male rats have been carried out in the past, for example, Gossell and Williams et al. (2006) argued that pumpkin seeds can inhibit and treat testosterone caused by prostate hyperplasia (17). Dreikorn (2002) also suggested that pumpkin seeds are useful for treating disorders of lower urinary tract and benign prostate hyperplasia (34).

Results of Hishefield (1991) and Konon et al. (2007) indicated that apoptosis in granulosa cells is the main atretic mechanism of ovarian follicles (35,36). Gossell-Williams et al. (2006) demonstrated the significant suppression of atretic follicle in consumers of pumpkin seeds (29). Our study did not confirm this and no significant variation in atretic follicles was seen in various mature groups. Thus, it can be reminded that the FSH level was enhanced in group received maximum dose (group 3); however no significant variation was seen in atretic follicles. These results are compatible with Case et al. (1998) who showed that pituitary gonadotropins are able to set directly follicular apoptosis; in this study, FSH suppressed follicular apoptosis as large as 60-62% [58]. It seems that several other factors are involved in this process which they need further studies in future.

The loss of the corpus luteum is a natural and necessary mechanism in the reproductive cycle which indicated the reduced cell performance. The results gained from counting ovarian corpus luteums in experimental groups of this study did not show any significant variation in contrast to the control group.

In general, results of this study indicated that hydro alcoholic extract of pumpkin seeds increases level of FSH and LH which in turn affect positively pituitary-ovaries axis and help considerably the oogenesis procedure. Further studies for finding the mechanism and the how this substance may affect are suggested. These studies are necessary for finding an alternative medicine for sexual hormones which are useful for treating infertility.

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بررسی اثر عصاره هیدرو الکلی تخمه کدو بر اووژنز و محور هورمونی هیپوفیز تخمدان در موش های صحرایی بالغ

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مجله پزشکی هرمزگان سال نوزدهم شماره دوم ۹۴ صفحات ۱۰۲-۹۲

چکیده

مقدمه: *تخمه کدو به عنوان یک داروی انرژی زا و تقویت کننده قوای جنسی در طب سنتی به طور رایج استفاده می‌شود. با توجه به در دسترس نبودن شواهد علمی کافی در خصوص میزان تأثیر این ماده بر اووژنز و محور هورمونی هیپوفیز تخمدان این مطالعه طراحی و اجرا گردید. آشکار ساختن اثر عصاره هیدرو الکلی تخمه کدو بر اووژنز و محور هورمونی هیپوفیز تخمدان موش‌های صحرایی بالغ.*

روش کار: *این مطالعه تجربی بر روی ۳۰ سر موش صحرایی ماده بالغ از نژاد ویستار(محدوده وزنی ۱۰±۱۸۰ گرم) انجام شد، که به طور تصادفی به ۴ گروه ۶ تایی: سه گروه تجربی و یک گروه کنترل تقسیم شدند. به گروه‌های تجربی عصاره (با دوزهای ۵۰، ۱۰۰، ۲۰۰ میلی‌گرم بر کیلوگرم وزن بدن عصاره هیدروالکلی تخمه کدو تنبل) به مدت ۲۱ روز متوالی به صورت درون صفاقی تزریق شد. یک روز بعد از آخرین تزریق خون‌گیری از موش‌ها جهت بررسی هورمون‌های جنسی به عمل آمد و تخمدان آنها نیز برای مطالعات بافت شناسی برداشته شد.*

نتایج: *تزریق عصاره هیدرو الکلی تخمه کدو تنبل به طور معنی‌داری باعث افزایش میزان سرمی هورمون‌های استروژن پروژسترون، LH و FSH و تعداد فولیکول‌های گراف و کاهش معنی‌دار در وزن تخمدان‌های چپ و تعداد فولیکول‌های بدوی، اولیه و ثانویه در گروه‌هایی تجربی نسبت به گروه شم و کنترل گردید (P< ۰/۰۵).*

نتیجه‌گیری: *تزریق عصاره هیدرو الکلی تخمه کدو باعث افزایش معنی‌دار در روند اووژنز و هورمون‌های جنسی LH ،FSH استروژن و پروژسترون می‌شود.*

کلیدواژه‌ها: *اووژنز – LH – FSH – استروژن – پروژسترون*

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